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Ameliorative Effects of N-acetylcysteine on the Expression of Oxidative Stress-Related Genes and Inflammatory Biomarkers in the Cardiac Tissues of Rats Exposed to Lead

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Abstract

Background: Lead, as the most important toxic heavy element, has several devastating effects on human health and influences most biochemical and physiological functions. It is widely accepted that lead can adversely affect the cardiovascular system since it can be quickly absorbed and recycled in the blood strain.

Objectives: This survey scrutinized the effects of N-acetylcysteine (NAC) on the oxidative damage, inflammation, and expression of protein kinase C-alpha (PKC-α) and ankyrin repeat domain 1 (ANKRD1) genes in the heart tissue of rats exposed to lead (Pb).

Methods: The rats were incidentally divided into five groups, including four study groups for the investigation of the effects of the single and continuous doses of lead were examined with and without NAC and a control group (G1). The levels of malondialdehyde (MDA), total antioxidant capacity (TAC), interleukin (IL)-10, and tumor necrosis factor alpha (TNF-α) were analyzed. A reverse transcription polymerase chain reaction was applied to investigate the expression of PKC-α and ANKRD1 genes.

Results: Continuous exposure to Pb significantly decreased serum levels of TAC and IL-10; however, it increased MDA and TNF-α contents ($P<0.001$). The continuous dose of Pb also dramatically increased the expression of PKC-α and ANKRD1 genes in the cardiac tissue by 4.27-fold and 3.07-fold, respectively ($P<0.001$). N-acetylcysteine treatments not only improved morphological changes, oxidative stress, and inflammatory biomarkers but also compensated antioxidant capacity and the expression of PKC-α and ANKRD1 genes in cardiac tissues.

Conclusion: Lead exposure is remarkably related to cardiotoxicity mainly by inducing oxidative stress, inflammation, and antioxidant discharge. N-acetylcysteine ameliorates Pb-induced cardiotoxicity by improving the antioxidants capacity, mitigating oxidative stress, and down expressing PKC-α and ANKRD1 genes.

Keywords: ANKRD1 genes, Antioxidants, Cardiac tissue, Lead, N-Acetyl cysteine, PKC-α genes

1. Background

Cardiovascular diseases are classified among the major causes of death in numerous developed and under developing countries (1). Recent evidence has revealed that smoking, obesity, sedimentary lifestyle, atherosclerotic lipid pattern, and arterial hypertension are the major risk factors correlated to cardiovascular diseases (2, 3). Nevertheless, there are numerous risk factors that their mechanism of action on cardiac function is not fully explained. Heavy metals, such as lead (Pb) and cadmium (Cd) are the most abundant xenobiotics in the human environment. These heavy metals are widely widespread in our environments, such as air, water, house dust, soil, and consumer products (4, 5). A large body of documents reported an interconnection between long exposure to Pb and different complications, including neurological disorders, hemolytic anemia, bone damage, liver and renal dysfunctions, cancers, and infertility (5-9). The results of recent studies have indicated that chronic exposure to Pb exposure may be the main risk factor for cardiac failure (10, 11); however, the exact mechanism by which Pb affects the cardiovascular system is not well-understood (12).

Although the knowledge on the effects of low Pb exposure on the cardiac system is incomplete, it is assumed that a change in the expression pattern of several genes involved in oxidative stress and inflammation is likely the main reason for Pb-induced cardiotoxicity (13). Oxidative stress is a condition in which an overabundance of free radicals is followed by a massive decrease in antioxidant capacity, and consequently, the occurrence of cells apoptosis. (14, 15). As a cardiac-specific stress-response protein, ankyrin repeat domain 1 (ANKRD1) is reported to be highly induced in different cardiomyopathies when mutated or overexpressed (16). In cardiac tissues, the overexpression of ANKRD1 in response to different stimuli has been shown to induce inhibitory effects on cardiomyocyte gene expressions (17). Protein kinase C (PKC) is a family of serine/threonine kinases that plays pleiotropic roles in the control of

numerous physiological and pathological responses, especially cardiac responses (18, 19). Dysregulation of PKC expression has been reported to be associated with heart failure (20). The findings of recent studies have demonstrated that PKC is sensitively activated by oxidative stress and inflammation (19, 21). Therefore, in the present study, it was predicted that Pb-induced cardiotoxicity might be mediated through oxidative stress, inflammation, and consequently, dysregulation of PKC and ANKRD1 genes. To support this theory, the expression pattern of these two genes, oxidative stress, and inflammation were designed to be investigated in the cardiac samples of rats exposed to Pb.

Since the imbalance between the production and detoxification of free radicals and inflammation is considered as a main mechanism of Pb-cardiotoxicity, antioxidant administration may prosper these irregularities through the prevention of oxidative stress. Protein kinase C-dependent cellular responses can also be prohibited using antioxidants. Consequently, PKC can be considered a substantial target for redox modifications by oxidants and antioxidants (21). A huge number of studies have been conducted on the anti-inflammatory effects of N-acetylcysteine (NAC) as a strong antioxidant. (22, 23). Given the destructive properties of Pb, it appears that oxidative damage and alterations of PKC and ANKRD1 genes expression may be a pivotal mechanism of its cardiotoxicity. The anti-inflammatory and modulating effects of NAC have been widely documented, showing that its administration is associated with a decrease in oxidative damage and the expression of PKC and ANKRD1 genes in the cardiac tissue. Therefore, this study was designed to search the effect of Pb on the oxidative stress biomarkers (e.g., total antioxidant and malondialdehyde [MDA] levels), inflammation (e.g., interleukin [IL]-10 and tumor necrosis factor [TNF]-α), and the expression of PKC and ANKRD1 genes in the cardiac tissue of Pb-treated rats. The healing impact of NAC on these parameters would also be considered.

2. Objectives

This survey scrutinized the effects of N-acetylcysteine (NAC) on the oxidative damage, inflammation, and expression of protein kinase C-alpha (PKC-α) and ankyrin repeat domain 1 (ANKRD1) genes in the heart tissue of rats exposed to lead (Pb).

3. Methods

3.1. Animals and treatments

Animals were purchased from the Pasteur Institute of Iran (Tehran). All treatments and tests were carried out on male Wistar rats (150-200 g body weight) that were housed under standard conditions (at the temperature of 22±2°C, humidity of 50%±5%, and 12-hour photoperiod cycles). The rats were fed with a standard diet (Javaneh Khorasan, Iran) and tab water throughout the study. In the current study, 30 animals were accidentally divided into five groups of six rats as follows:

Group 1 (G1): control, received no treatment;

Group 2 (G2): received only a single dose of Pb (70 mg/kg) on the first day of the study;

Group 3 (G3): received a continuous dose of Pb (2 mg/kg) every day for 4 weeks;

Group 4 (G4): administrated with Pb (70 mg/kg) and NAC (50 mg/kg) on the 1st day of the experiment; and

Group 5 (G5): treated with Pb (2 mg/kg) and NAC (50 mg/kg) solutions every day for 4 weeks.

Lead acetate (Pb(CH3COO)2) and NAC were purchased from Sigma-Aldrich (USA) and Albertsons (USA), respectively. In G4 and G5, Pb and NAC were administrated at 3-hour intervals to avoid the formation of unwanted complexes. The final volume of treatments was 1 cc, which was administrated orally (gavage).

3.2. Sample collection

The rats were anesthetized with a combination of xylasine (10 mg/kg) and ketamine (30-50 mg/kg) 48 hours after the last treatment. After opening the chest, blood samples were collected from the abdominal aorta for the evaluation of serum levels of Pb and inflammatory mediators. For histological analysis, fractions of cardiac tissue were removed and stored in 10% formalin for 2 weeks. Following the dehydrating process, tissues were embedded in paraffin, sectioned at 5 µm thickness using a manual rotary microtome (Biobase, Shandong. Co, China), and stained with hematoxylin-eosin (24). The sections were then analyzed for histopathological alterations under the light microscope (Kern & Sohn, Germany). To study the expression pattern of related genes, a piece of heart tissue (~300 mg) was removed and homogenized (Hielscher, UP100H, Germany) in phosphate buffer (with pH 7.0) at 4°C. Subsequently, the homogenized tissue was centrifuged (Hettich, Germany) at 4500×g for 15 min at 4 °C (25). The supernatants were rolled up and immediately stored at -80°C with ribonucleic acid (RNA) later solution (Sinaclon, Iran).

3.3. Oxidative stress and inflammatory biomarkers

Serum total antioxidant capacity (TAC) was measured by ferric reducing antioxidant power (FRAP) assay, as previously described by Benize et al. (26). The levels of MDA were assayed by a specific colorimetric kit (ZB-0156-R9648, Germany) according to the manufacturer's protocol. Glutathione (GSH) level was measured according to the Tietz method (27). The liver TNF-α and IL-10 contents were assessed using the Rat enzyme-linked immunosorbent assay kits,

manufactured by ZellBio Company (ZB-0764-R9648 and ZB-0522-R9648, respectively).

3.4. Lead Measurement

For the Pb analyses, 1 ml of blood samples were centrifuged at 600 × g for 10 min. Supernatants were then diluted 5-fold using deionized water. Serum Pb concentrations were measured by atomic absorption spectroscopy (Perkin Elmer model 2380, USA). The standard curve was plotted using different concentrations of Pb (0.01-0.8 mg/l).

3.5. Gene expression analysis

Total RNA was extracted (RNX-Plus, Sinaclon; RN7713C, Iran) from homogenized cardiac samples. Extracted RNAs were confirmed for their quality using a Nanodrop ND-1000 spectrophotometer (Thermo Sci, USA). RevertAid First Strand cDNA Synthesis Kit (Thermo Science, Germany) and random hexamer primers (Thermo science, Germany) were applied for complementary DNA synthesis at 42oC for 1 h. Amplifications were performed by a Rotor-Gene 6000 (Corbett Research, Australia) thermocycler in 40 cycles. The amounts of 5 µl of the master mix and 100 nM of primers were applied for each reaction. Primer sequences were as follows: PKC-α, 5/- CAA-GCA-GTG-CGT-GAT-CAA-TGT-3/ (forward), 5/- GGT-GAC-GTG-CAG-CTT-TTC-ATC-3/ (reverse); ANKRD1, 5/- GCTGGAGC CCAGATTGAA-3/ (forward), 5/- CTCCACGACAT GCCCAGT-3/ (reverse); and glyceraldehyde 3-phosphate dehydrogenase, 5/-AAGTTCAACGGC ACAGTCAAGG-3/ (forward); 5/-CATACTCAGCAC CAGCATCACC-3/ (reverse). Glyceraldehyde 3-phosphate dehydrogenase was used as a reference gene for the normalization of the levels of messenger RNA. The expression of relative genes was assessed using the 2-ΔCt method.

3.6. Statistical analysis

The collected data were analyzed in SPSS software (version 20) using one-way ANOVA and post-hoc Tukey test. Moreover, the means of all quantitative data were compared with normal distribution between groups. A p-value of < 0.05 was considered significant.

4. Results

Histopathological analysis of the heart tissue between different groups revealed numerous inflammatory aggregations, degeneration, and necrosis in most myocardial muscle bundles in the G2 and G3, compared to the control group (G1). Although the rate of necrosis was significant in the G3, the single dose of Pb caused a wide inflammatory response in G2. Compared to G2 and G3, NAC treatment significantly decreased inflammatory cells and necrosis rate respectively in the G4 and G5 (Figure 1).

The means of oxidative stress and inflammatory

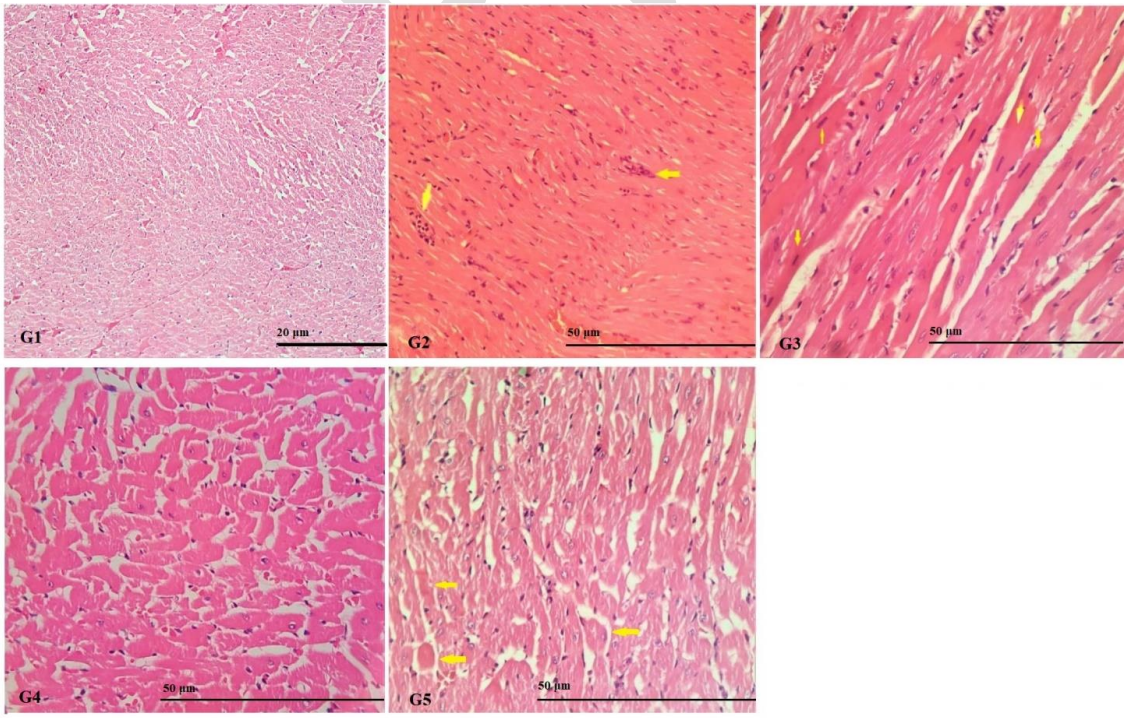

Figure 1. Histopathological alterations between different groups
While a single dose of Pb induced inflammatory aggregations in the heart tissue in the G2, continuous dose increased the rate of Necrosis in the G3. However, NAC treatment almost reversed these alterations in the G4 and G5.

Table 1. Comparison of oxidative stress biomarkers between different groups

	G1	G2	G3	G4	G5	P-value
FRAP (µg/ml)	529.6±75.18	514.83±38.87	247.19±38.54*	518.11±41.0	394.22±51.48**	<0.001
MDA (µg/ml)	12.41±2.02	14.26±2.48	47.82±6.38*	12.16±1.85	29.12±4.69**	<0.001
IL-10 (pg/mg protein)	15.32±3.61	14.69±3.06	7.69±2.05*	14.93±4.18	11.2±2.15**	<0.001
TNF-α (pg/mg protein)	16.14±3.71	15.83±4.6	35.23±6.72*	16.08±2.94	22.36±3.55**	<0.001

G1: Control; G2: Single dose of Pb; G3: Continuous dose of Pb; G4: Single dose of Pb + N-acetylcysteine; G5: Continuous dose of Pb + N-acetylcysteine
FRAP: Ferric reducing antioxidant power; MDA: Malondialdehyde; IL: Interleukin; TNF: Tumor necrosis factor
*P<0.001 and **P<0.01, compared to the control group

biomarkers are presented in Table 1. Rats affected by the continuous dose of Pb exhibited lower mean values of FRAP (247.19±38.54 µg/ml) and IL-10 (7.69±2.05 pg/mg protein), in comparison with other groups (P<0.001). It was revealed that NAC therapy significantly recovered FRAP (from 247.19±38.54 µg/ml to 394.22±51.48 µg/ml; P=0.011) and IL-10 (from 7.69±2.05 pg/mg to 11.2±2.15 protein; P=0.039) values in rats with continuous exposure to Pb. The means of serum MDA and TNF-α contents were significantly higher in rats exposed to a continuous dose of Pb (P<0.001) than in the other groups. N-acetylcysteine treatments significantly decreased the mean values of MDA (from 47.82±6.38 µg/ml to 29.12±4.69 µg/ml; P=0.009) and TNF-α (from 35.23±6.72 pg/mg to 22.36±3.55 pg/mg protein; P=0.028) in continuous lead-exposed rats. Oxidative and inflammatory biomarkers did not change in the G1, G2, and G4 groups.

Lead levels analysis in serum samples of all groups is shown in Figure 2. In rats exposed to a continuous dose of Pb, the mean level of Pb (1.94±0.18 µg/l) significantly increased by about 295.9%, compared to the control group (0.49±0.062 µg/l). It was also found that NAC treatments significantly decreased serum Pb levels of rats that continuously treated with Pb by approximately 50.5% (from 1.94±0.18 µg/l to 0.96±0.12 µg/l; P<0.01).

A significant difference was observed in the expression patterns of ANKRD1 (Figure 3) and PKC-α (Figure 4) genes between groups (P<0.001). Overall, continuous exposure to Pb caused a significant increase in ANKRD1 (3.07-fold) and PKC-α (4.27-fold) genes, in comparison to the control group (Figure 2; P<0.001). In contrast, NAC supplementation treatment significantly refined the expression of these genes; accordingly, NAC significantly decreased the expression of ANKRD1 and PKC-α genes by 1.81-fold and 1.26-fold, respectively.

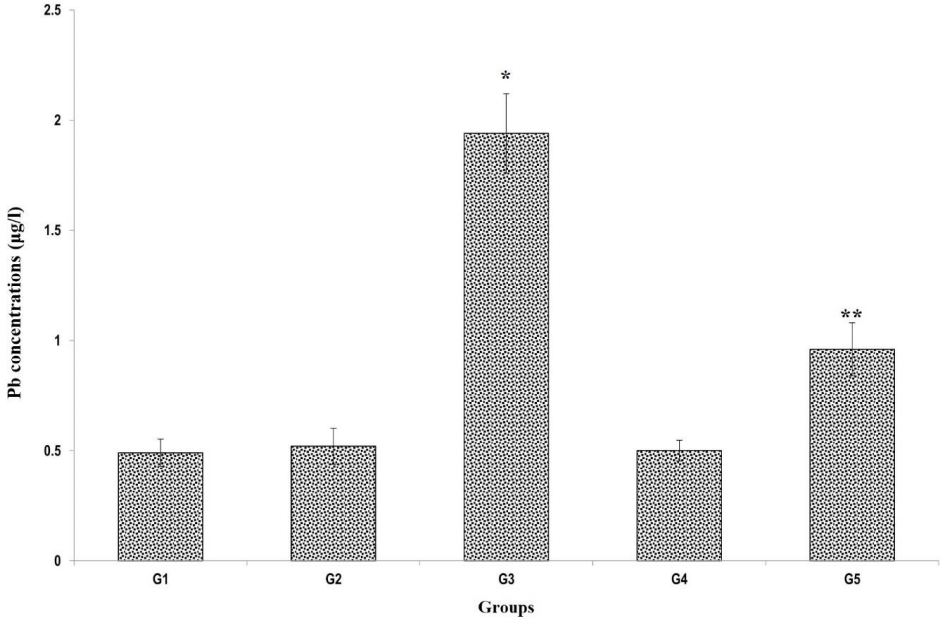

Figure 2. Comparison of the mean of Pb level between different groups
G1: Control; G2: Single dose of Pb; G3: Continuous dose of Pb; G4: Single dose of Pb + N-acetylcysteine; G5: Continuous dose of Pb + N-acetylcysteine
*P<0.001 and **P<0.01, compared to the control group; Error bars are presented as standard error.

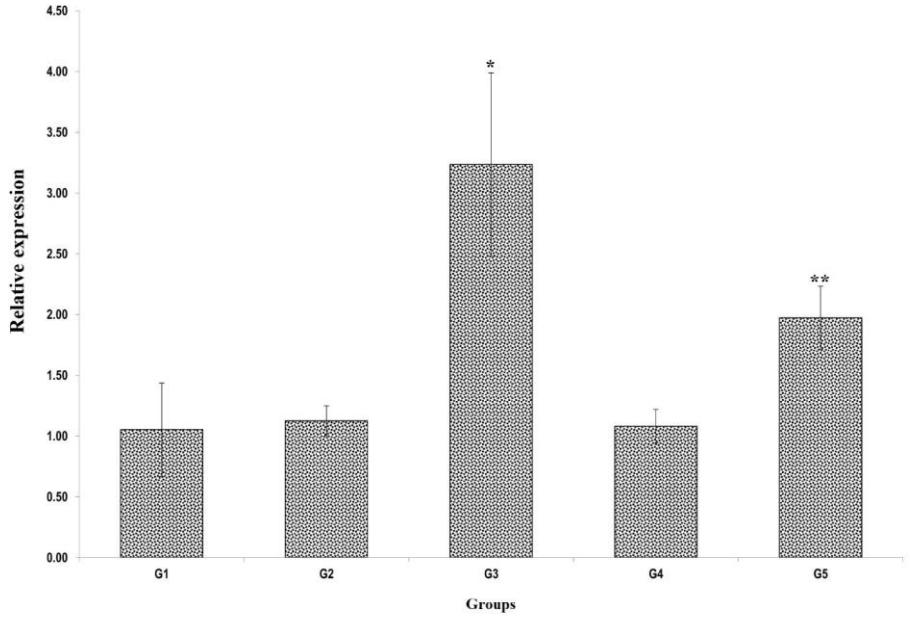

Figure 3. Comparison of the mean messenger RNA levels of *ANKRD1* gene between groups
G1: control; G2: Single dose of Pb; G3: Continuous dose of Pb; G4: Single dose of Pb + N-acetylcysteine; G5: Continuous dose of Pb + N-acetylcysteine
*P<0.001 and **P<0.01, compared to the control group. Error bars are presented as standard error

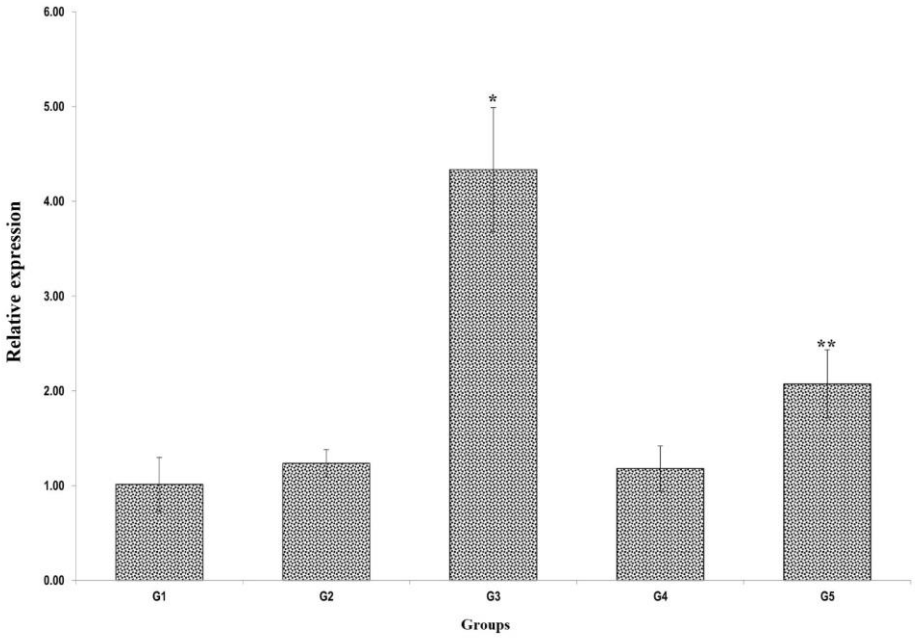

Figure 4. Comparison of the mean messenger RNA levels of PKC-α gene between groups
G1: control; G2: Single dose of Pb; G3: Continuous dose of Pb; G4: Single dose of Pb + N-acetylcysteine; G5: continuous dose of Pb + N-acetylcysteine
*P<0.001 and **P<0.01, compared to the control group. Error bars are presented as standard error

5. Discussion

The toxicity of Pb on cardiac function has raised wide public concern worldwide. The current study represented the potential role of NAC on histological alterations, oxidative stress, inflammation biomarkers, and the gene expression of ANKRD1 and PKC-α in the cardiac tissue of rats exposed to Pb. Our results showed that Pb exposure, especially in the chronic phase, was significantly correlated to heart tissue damage, oxidative stress, and inflammatory mediators. Histopathological and morphological examinations revealed that continuous exposure to Pb caused dramatic cardiac and vascular damage. It

was also found that exposure to Pb led to a significant reduction in antioxidants capacity and an increase in lipid peroxidation levels among exposed animals. Repeatedly exposure to Pb was significantly correlated with Pb accumulation in the blood of exposed animals. Moreover, the ongoing application of Pb significantly provoked the overexpression of ANKRD1 and PKC-α genes in the cardiac tissue of polluted rats. It has been reported that the overexpression of PKC-α can be linked to higher degrees of oxidative stress and inflammation and it is implicated in the neurotoxicity by Pb, while ANKRD1 overexpression may increase the risk of lead-induced cardiomyopathy (28). Increased levels of oxidative stress not only decline the effective concentration of some essential antioxidants but also cause severe damages to various tissues or organs (29-31). The results of a previous study have shown that Pb toxicity is correlated to the elevated activation of PKC in the brain of rats (32). Hwang et al. found that PKC was extremely inflated in lead workers (28). More recently, Hegde et al. reported a 39-year-old man with cardiomyopathy of persistent lead exposure (33). They concluded that continual Pb exposure had direct toxic effects on the myocardium and enhanced the risk of cardiomyopathy. There are several previous reports showing a higher risk of myocarditis due to chronic lead exposure in humans (34, 35). Another previous study compared lead factory staff with healthy individuals (36). The results of the mentioned study showed that chronic exposure to Pb significantly affected the left ventricular in the group with lead exposure. Although the findings of a growing number of studies have reported the cardiotoxicity of Pb exposure, the exact mechanism in which Pb affects cardiac function has remained unclear. Based on the results of our study, oxidative stress, inflammation, and overexpression of ANKRD1 and PKC-α genes probably was a principal cause of Pb-induced cardiotoxicity in exposed rats. To support our idea, the findings of numerous studies have previously indicated genotoxicity, cytotoxicity, and inflammatory effects of Pb on different tissues. The results of a recent study revealed a significant reduction in antioxidant enzymes, such as superoxide dismutase (SOD), and catalase activity after being exposed to Pb (37). Offor et al. demonstrated that Pb exposure significantly enhanced the MDA level; however, it significantly reduced the activity of SOD, glutathione peroxidase (GPX), and total GSH (38). Kumar et al. found that although Pb exposure significantly increased MDA contents, it reduced the mean level of GSH in the liver of poultries (39). According to the findings of recent research, blood Pb level was significantly higher (~4-5 times) in workers who were in close contact with Pb than in normal individuals (40). Furthermore, a significant decrease in plasma levels of GSH and TAC, however, increased MDA and hydrogen peroxide contents were found in these cases (40). Altogether, these data indicate that Pb-induced oxidative stress and inflammation is a main mechanism of Pb toxicity that is subsequently associated with cardiac cells injury and apoptosis. Xu et al. found that Pb exposure induced histone acetylation, and consequently, vascular and cardiac cells apoptosis (41).

Based on these observations and concepts around Pb-induced cardiotoxicity, antioxidants may have wisely been considered for the protection of cardiac against oxidative stress and inflammation. Here, we evaluated the effect of NAC supplementation to mitigate morphological alterations, oxidative stress, inflammation, and overexpression of ANKRD1 and PKC-α genes caused by Pb exposure. In our study, it was demonstrated that NAC treatment not only declined cell injuries and TNF-α and MDA contents but also increased total antioxidant capacity and IL-10. It was also revealed that NAC attenuated the expression of ANKRD1 and PKC-α genes in the heart tissue of Pb-treated rats. Although the levels of oxidative stress and expression of ANKRD1 and PKC-α genes were somewhat high in the cardiac tissue of rats treated with a repeated dose of Pb and NAC, these abnormalities were considerably compensated in this group compared to the rats that were only treated with a chronic dose of Pb. In line with these findings, there are plenty of documents reporting that NAC therapy decreases reactive oxygen species (ROS) production and inflammatory response in various tissues. Wang et al. revealed that NAC treatment could mitigate oxidative stress and restore the GSH-related enzymes activities in vitro (42). Shieh et al. have illustrated that NAC supplementation reversed malathion-induced oxidative stress and decreased the expression of apoptotic biomarkers, such as Bax, Bcl2, Caspases-3, and Caspases-9, in human astrocytes (43). The results of a further study conducted by Chen et al. (44) showed that in cadmium-affected rats, NAC treatment significantly prevented ROS production, brain damage, and apoptosis mainly by increasing the activities of Cu/Zn-SOD, catalase, GSH, and GPX enzymes in the brain tissue, which was consistent with our findings. According to former accomplished data and current results, oxidative stress and inflammation play the central role in cardiotoxicity of Pb, and NAC supplementation reduces the genotoxicity and cytotoxicity effects of Pb in heart tissue.

6. Conclusion

In conclusion, our results indicated that Pb exposure, especially in the chronic phase, was firmly connected with Pb deposition, morphological changes in cardiac tissue, oxidative stress, antioxidant depletion, inflammation, and the overexpression of ANKRD1 and PKC-α genes in the heart tissue. It was also found that NAC could protect cardiac tissues

against Pb toxicity by down-regulating these genes, elevating antioxidants capacity, and mitigating oxidative stress and inflammation.

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Footnotes

Conflicts of interest: The authors confirm that they have no conflict of interest.
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References

1. Vaziri ND, Gonick HC. Cardiovascular effects of lead exposure. *Indian J Med Res*. 2008; **128**(4):426-35. [PMID: 19106438]
2. Poręba R, Gać P, Poręba M, Andrzejak R. Environmental and occupational exposure to lead as a potential risk factor for cardiovascular disease. *Environ Toxicol Pharmacol*. 2011;**31**(2):267-77. doi: 10.1016/j.etap.2010.12.002
3. Hanke W, Szeszenia-Dabrowska N. The role of long-term exposure to lead in the pathogenesis of hypertension--review of epidemiologic studies. *Medycyna Pracy*. 1994;**45**(2):163-70. [PMID: 8007826]
4. da Silva RF, Borges Cdos S, Villela ESP, Missassi G, Kiguti LR, Pupo AS, et al. The Coadministration of N-Acetylcysteine Ameliorates the Effects of Arsenic Trioxide on the Male Mouse Genital System. *Oxid Med Cell*. 2016;**2016**:4257498. [PMCID: PMC4709715] doi: 10.1155/2016/4257498
5. Hou S, Yuan L, Jin P, Ding B, Qin N, Li L, et al. A clinical study of the effects of lead poisoning on the intelligence and neurobehavioral abilities of children. *Theor Biol Med Model*. 2013;**13**:10-17. [PMCID: PMC3598508] doi: 10.1186/1742-4682-10-13
6. AG. V. Hemopoietic, hemostatic and mutagenic effects of lead and possible prevention by zinc and vitamin C. *Al Ameen J Med Sci*. 2009;**2**:27–36. https://www.cabdirect.org/cabdirect/abstract/20103114254
7. Grant LD. Environmental Toxicants. John Wiley & Sons. *Lead And Compounds*. 2020. doi: 10.1002/9781119438922.ch17.
8. Navas-Acien A, Guallar E, Silbergeld EK, Rothenberg SJ. Lead exposure and cardiovascular disease--a systematic review. *Environ Health Perspect*. 2007;**115**(3):472-82. [PMCID: PMC1849948] doi: 10.1289/ehp.9785
9. Flora SJS, Pachauri V, Saxena G. Academic Press. Arsenic, cadmium and lead. *Reprod Devel Toxicol*. 2011;**1**:415–438. doi: 10.1016/B978-0-12-804239-7.00031-7.
10. Ari E, Kaya Y, Demir H, Asicioglu E, Keskin S. The correlation of serum trace elements and heavy metals with carotid artery atherosclerosis in maintenance hemodialysis patients. *Biol Trace Elem Res*. 2011;**144**(1-3):351-9. doi: 10.1007/s12011-011-9103-0
11. Chlebda E, Antonowicz-Juchniewicz J, Andrzejak R. The effect of occupational exposure to heavy metals and arsenic on serum concentrations of carotenoids in copper foundry workers. *Med Pr*. 2004;**55**(5):389-401. [PMID: 15768892]
12. Kuliczkowski W, Jołda-Mydłowska B, Kobusiak-Prokopowicz M, Antonowicz-Juchniewicz J, Kosmala W. Effect of heavy metal ions on function of vascular endothelium in patients with ischemic heart disease. *Pol Arch Intern Med*. 2004;**111**(6):679-85. [PMID: 15508790]
13. Beigi Harchegani A, Dahan H, Tahmasbpour E, Bakhtiari Kaboutaraki H, Shahriary A. Effects of zinc deficiency on impaired spermatogenesis and male infertility: the role of oxidative stress, inflammation and apoptosis. *Hum Fertil*.. 2020;**23**(1):5-16. doi: 10.1080/14647273.2018.1494390
14. Flora G, Gupta D, Tiwari A. Toxicity of lead: A review with recent updates. *Interdiscip Toxicol*. 2012;**5**(2):47–58. [PMCID: PMC3485653] doi: 10.2478/v10102-012-0009-2
15. Mirnamniha M, Faroughi F, Tahmasbpour E, Ebrahimi P, AB. H. An overview on role of some trace elements in human reproductive health, sperm function and fertilization process. *Rev Environ Health*. 2019;**34**:339-48. doi: 10.1515/reveh-2019-0008
16. Moulik M, Vatta M, Witt SH, Arola AM, Murphy RT, McKenna WJ, et al. ANKRD1, the gene encoding cardiac ankyrin repeat protein, is a novel dilated cardiomyopathy gene. *J Am Coll Cardiol*. 2009;**54**(4):325-33. [PMCID: PMC2915893] doi: 10.1016/j.jacc.2009.02.076
17. Mikhailov AT, Torrado M. The enigmatic role of the ankyrin repeat domain 1 gene in heart development and disease. *Int J Dev Biol*. 2008;**52**(7):811-21. doi: 10.1387/ijdb.082655am
18. Steinberg SF. Structural basis of protein kinase C isoform function. *Physiol Rev*. 2008;**88**(4):1341-78. [PMCID: PMC5635086] doi: 10.1007/s10741-017-9634-3
19. Palaniyandi SS, Sun L, Ferreira JC, Mochly-Rosen D. Protein kinase C in heart failure: a therapeutic target? *Cardiovasc Res*. 2009; **82**(2):229-39. [PMID: 19168855] doi: 10.1093/cvr/cvp001.
20. Singh RM, Cummings E, Pantos C, Singh J. Protein kinase C and cardiac dysfunction: a review. *Heart Fail Rev*. 2017;**22**(6):843-59. [PMID: 19168855] doi: 10.1093/cvr/cvp001
21. Gopalakrishna R, Jaken S. Protein kinase C signaling and oxidative stress. *Free Radic Biol*. 2000;**28**(9):1349-61. doi: 10.1016/s0891-5849(00)00221-5
22. Pudari H, et al. Evaluation of protective effect of N-acetyl cysteine on arsenic-induced hepatotoxicity. *Nat Sci Biol Med*. 2013;**4**:393–5. [PMCID: PMC3783787] doi: 10.4103/0976-9668.116986
23. Dekhuijzen PN, et al. Antioxidant properties of N-acetylcysteine: their relevance in relation to chronic obstructive pulmonary disease. *Eur Respir J*. 2004;**23**:629-36. doi: 10.1183/09031936.04.00016804
24. Molh AKS, Ting LC, Khan J, Al-Jashamy K, Jaafar H, Islam MN. Histopathological studies of cardiac lesions after an acute high dose administration of methamphetamine. *Malays J Med Sci*. 2008;**15**(1):23-31. [PMID: 22589611]
25. Ma Z, Chu L, Liu H, Wang W, Li J, Yao W, et al. Beneficial effects of paeoniflorin on non-alcoholic fatty liver disease induced by high-fat diet in rats. *Sci Rep*. 2017;**7**:44819. [PMCID: PMC5353673] doi: 10.1038/srep44819
26. Benzie IF. Lipid peroxidation: a review of causes, consequences, measurement and dietary influences. *International Food Sci Nutr*. 1996;**47**(3):233-61. doi: 10.3109/09637489609012586
27. Faco. T. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. *Anal Biochem*. 1969;**27**:502–22. doi: 10.1016/0003-2697(69)90064-5
28. Hwang KY, Lee BK, Bressler JP, Bolla KI, Stewart WF, Schwartz BS. Protein kinase C activity and the relations between blood lead and neurobehavioral function in lead workers. *Environ Health Perspect*. 2002;**110**(2):133-8. [PMCID: PMC1240726] doi: 10.1289/ehp.02110133
29. Hosseinzadeh Colagar A, Pouramir M, Tahmasbpour Marzony E, Jorsaraei SGA. Relationship between seminal malondialdehyde levels and sperm quality in fertile and infertile men. *Braz Arch Biol Technol*. 2009;**52**:1387-92. doi: 10.1590/S1516-89132009000600010
30. Hosseinzadeh Colagar A, M Pouramir, Tahmasbpour E. Seminal plasma total antioxidants capacity of the infertile smoker and nonsmoker men. *Shahid Cham Uni Sci*. 2008;**19**:124-31.
31. Colagar AH, Marzony ET, Chaichi MJ. Zinc levels in seminal plasma are associated with sperm quality in fertile and

infertile men. *Nutr Res Rev*. 2009;**29**(2):82-88. doi: 10.1016/j.nutres.2008.11.007
32. Long GJ, Rosen JF, Schanne FA. Lead activation of protein kinase C from rat brain. Determination of free calcium, lead, and zinc by 19F NMR. *J Biol Chem*. 1994;**269**(2):834-7. doi: 10.1016/S0021-9258(17)42188-0.
33. Hegde S, Maysky M, Zaidi A. A Rare Case of Lead-Induced Cardiomyopathy. *JACC Case Reports*. 2020;**2**(10):1496-500. PMCID: PMC8302118 doi: 10.1016/j.jaccas.2020.05.047
34. Read JL, Williams JP. Lead myocarditis: report of a case. *Am Heart J*. 1952;**44**(5):797-802. doi: 10.1016/0002-8703(52)90107-5
35. Freeman R. Reversible Myocarditis Due to Chronic Lead Poisoning in Childhood. *Arch Dis Child*. 1965;**40**:389–93. [PMCID: PMC2019279] doi: 10.1136/adc.40.212.389
36. Kasperczyk S, Przywara-Chowaniec B, Kasperczyk A, Rykaczewska-Czerwińska M, Wodniecki J, Birkner E, et al. Function of heart muscle in people chronically exposed to lead. *Ann Agric Environ Med*. 2005;**12**(2):207-10. [PMID: 16457475]
37. Elgawish RAR, Abdelrazek HMA. Effects of lead acetate on testicular function and caspase-3 expression with respect to the protective effect of cinnamon in albino rats. *Toxicol Rep*. 2014;**1**:795-801. [PMID: PMC5598148] doi: 10.1016/j.toxrep.2014.10.010
38. Offor SJ, Mbagwu HO, Orisakwe OE. Improvement of Lead Acetate-Induced Testicular Injury and Sperm Quality Deterioration by Solanum Anomalum Thonn. Ex. Schumach Fruit Extracts in Albino Rats. *J Family Reprod Health*. 2019;**13**(2):98-108. doi: 10.18502/jfrh.v13i2.1916.
39. Kumar R, Reddy AG, Anjaneyulu Y, Reddy GD. Oxidative Stress Induced by Lead and Antioxidant Potential of Certain Adaptogens in Poultry. *Toxicol Int*. 2010;**17**:45–8. [PMCID: PMC2997453] doi: 10.4103/0971-6580.72668
40. Shraideh Z, Badran Z, Hunaiti A, Battah A. Association between occupational lead exposure and plasma levels of selected oxidative stress related parameters in Jordanian automobile workers. *Int J Occup Med Environ Health*. 2018;**31**(4):517–525. doi: 10.13075/ijomeh.1896.01243
41. Li-Hui Xu, Fang-Fang Mu, Jian-Hong Zhao, Qiang He, Cui-Li Cao, Hui Yang. Lead Induces Apoptosis and Histone Hyperacetylation in Rat Cardiovascular Tissues. *PLoS ONE*. 2015;**10**:e0129091. PMCID: PMC4468051 doi: 10.1371/journal.pone.0129091
42. Wang J, Li M, Zhang W, Gu A, Dong J, Li J, et al. Protective Effect of N-Acetylcysteine against Oxidative Stress Induced by Zearalenone via Mitochondrial Apoptosis Pathway in SIEC02 Cells. *Toxins (Basel)*. 2018;**10**:407-411. [PMCID: PMC6215273] doi: 10.3390/toxins10100407
43. Shieh P, Jan CR, Liang WZ. The protective effects of the antioxidant N-acetylcysteine (NAC) against oxidative stress-associated apoptosis evoked by the organophosphorus insecticide malathion in normal human astrocytes. *Toxicology*. 2019;**417**:1-14. doi: 10.1016/j.tox.2019.02.004
44. Chen S, Ren Q, Zhang J, Ye Y, Zhang Z, Xu Y, et al. N-acetyl-L-cysteine protects against cadmium-induced neuronal apoptosis by inhibiting ROS-dependent activation of Akt/mTOR pathway in mouse brain. *Neuropathol Appl Neurobiol*. 2014;**40**:759-77. [PMID: PMC4043941] doi: 10.1111/nan.12103